Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 6A.38

IN VITRO MICROTUBERIZATION FOR SIMULATING THE DEVELOPMENTAL PHYSIOLOGY OF UNDERGROUND STORAGE ORGAN IN *HELIANTHUS TUBEROSUS*

GUTIERREZ PESCE P., BIZZARRI M., RUGINI E., DE PACE C.

Department of Science and Technologies for Agriculture, Forests, Nature, and Energy, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)

Nodal explants, cold treatment, sprouting, microtubers, Jerusalem artichoke

Production of *Helianthus tuberosus* L. (*Ht*) microtubers by tissue culture, might become a model for simulating the developmental physiology of underground storage organ formation, and a tool for early selection strategy for tuber traits. In particular, carbohydrate levels at microtuber physiological maturity may give insight on the possible relation to the carbohydrate content of mature tubers in the field. Other microtuber traits (time to induction, color, shape) might reflect the genetic diversity among clonal explants for the physiological ability to microtuber differentiation. Microtubers may also be considered as the material of choice for the greenhouse production of disease-free plantlets to propagate *Ht* clones. There are no reports on the proper procedure for *in vitro* microtuber induction in genetically different *Ht* clones, although a general protocol has been proposed.

The method described here allows microtuber induction on single nodal explants dissected from *in-vitro* grown plantlets from tubers of three *Ht* clones: Violet de Rennes (VR), Ungheria-3B (CU-3B), and K8-HS142. Field-grown tubers were surface sterilized with 0.5% sodium hypochlorite, rinsed with sterile water, sliced in disks containing at least one bud, and treated with a BAP/GA_3 solution at 0, 1, 10 and 50 ppm, and placed in the dark for two days. Emerged shoots were detached by cutting with a sterile blade and transferred to sterile jars containing autoclaved proliferation medium where they were subcultured until a constant rate of proliferation was reached. The proliferation medium consisted of Murashige and Skoog (MS) basal salts and vitamins, BAP 0.5 mg L^{-1}, NAA 0.05 mg L^{-1}, sucrose 3%, agar 0.6%, and activated charcoal at 1%. For the microtuberization trials, shoots were divided into segments containing one node each. Four microtuberization agarized media were tested; they consisted of MS basal salts and vitamins, at pH 8, each one containing one of the four combinations of BAP (0 and 0.5 mg L^{-1}) and sucrose (6% and 8%). The node explants of the three clones were subcultured in the four media and were maintained in the dark at 18°C until the microtubers appeared. Microtuberization took place with high success in the VR and CU-3B clones, and it was less conspicuous in the K8-HS142 clone. The presence of BAP in the media improved microtuber formation but it was not an effective factor for microtuber induction. Microtuberization occurred after 1.5 months on VR nodal explants, and after 2 and 2.5 months on the CU-3B and K8-HS142 explants, respectively. Microtubers formed on VR explants were violet in color and pear-shaped, beige and round for CU-3B, and beige and elongated for K8-HS142. Microtubers from K8-HS142 were susceptible to dehydration. The time elapsed for microtuber formation and their color and shape, paralleled the expression of similar traits for the tubers formed by the field-grown plants. Microtubers were stored for two months at +4°C, without loosing dormancy. They sprouted only with a treatment with 0.1% GA_3 for 10 min., without a cold

pretreatment. Microtubers from the control group (no cold pretreatment, no immersion in GA_3), expressed a significant lower percentage of sprouting. The plantlets, obtained from all the sprouted microtubers, displayed a normal phenotype, and when were transferred to greenhouse they continued to grow.